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Sangar, C. and Singh, Jaipaul ORCID: 0000-0002-3200-3949 (2016) Effects of Streptozotocin- induced Type I Diabetes Mellitus on Cation contents in Urinary Bladder tissues of the rat. International Journal of Pharmaceutical Sciences and Research, 7 (2). pp. 789-797. ISSN 2320-5148

It is advisable to refer to the publisher's version if you intend to cite from the work.
10.13040/IJPSR.0975-8232.7(2).789-97

IJPSR (2016), Vol. 7, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 September, 2015; received in revised form, 13 January, 2016; accepted, 16 January, 2016; published 01 February, 2016

EFFECTS OF STREPTOZOTOCIN- INDUCED TYPE I DIABETES MELLITUS ON CATION CONTENTS IN URINARY BLADDER TISSUES OF THE RAT

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Keywords:

Diabetes, Streptozotocin, Urinary Bladder, Cation Contents

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ABSTRACT: Diabetes mellitus is known to induce microvascular changes and alterations to neuronal functions. Diabetic neuropathy and diabetic nephropathy are two of the major long-term complications of diabetic mellitus (DM). The main aim of the comparative study is to investigate the role of cations contents compared to the age matched control.Adult rats were humanely killed and detrusor muscles of urinary bladder located and excised rapidly and placed in organ baths. Then urinary bladder detrusor muscles were blotted, weighed and dissolved in concentrated nitric acid for the measurements of cation contents. The levels of Na^+, K^+, Mg^{2+}, Zn^{2+}, Cu^{2+}, Ca^{2+}, Pb^{2+} and Fe^{2+} were measured by flame photometry, atomic absorbance spectrophotometry and inductively coupled plasma- mass spectrometry. The results show marked changes in the characteristics of diabetic and control animals. Diabetic rats weighed significantly ($P<0.05$) less compared to age-matched control rats. Diabetic rats also have significantly ($P<0.05$) elevated blood glucose, weight of bladder and bladder strip compared to age-matched control rats. The results also show that the levels of cations for Na^+, K^+, Ca^{2+} and Mg^{2+} were significantly ($P<0.05$) reduced in unstimulated diabetic urinary bladder than unstimulated control urinary bladder but for trace elements like Zn^{2+}, Cu^{2+} and Pb^{2+} the cations content increased in unstimulated diabetic urinary bladder than unstimulated control urinary bladder**.**Hence we conclude that these differences in the cation contents in STZ-induced DM results in malfunctioning of bladder and the development of long term complications of DM.

INTRODUCTION: Diabetes mellitus (DM) is a condition in which the pancreas no longer produces enough insulin or when cells stop responding to the insulin that is produced due to which glucose in the blood cannot be absorbed into the cells of the body this leads to hyperglycemia in the urine. [1] DM is a syndrome with metabolic, vascular, and neuropathic components that are all interrelated.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(2).789-97

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (2).789-97

Diabetes and urologic diseases are very common health problems that markedly increase in prevalence and incidence with advancing age. Diabetes is associated with an earlier onset and increased severity of urologic diseases that results in costly and debilitating urologic complications. Urologic complications, including bladder dysfunction, sexual and erectile dysfunction, as well as Urinary Tract Infections (UTIs), have a profound effect on the quality of life of men and women with diabetes. [2] Diabetes mellitus (DM) is a rampant epidemic worldwide. DM affects more than 285 million people worldwide in 2010 and it estimated that it would affect 439 million by the year 2030. [3] Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed

with the disease. [4] In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. [5] The prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease. [6]

Diabetes can be classified into two different forms- **Type I diabetes mellitus** also known as insulin-dependent diabetes (IDDM) or juvenile diabetes. **Type II diabetes mellitus** is known as non-insulin- dependent diabetes (NIDDM) or adult onset.[7]

The main aim of the comparative study is to investigate the role of cations contents compared to the age matched control.

MATERIALS AND METHODS:

Ethical approval:

All the experiments have a relevant clearance from Ethical Committee of the University of Central Lancashire for the use of animals in research.

Experimental procedure:

Animal model:

The experiments were carried out using adult male Wistar rats with body weights; 151.1 – 174.3 g. They were supplied by Animal Service of the University of Central Lancashire. Animals were divided into two equal groups: the control and diabetes groups.

Induction of Diabetes Mellitus:

To induce diabetes, an amount of 60 mg/kg streptozotocin (STZ) was dissolved in citrate buffer (0.3 mL) and administered as a single intraperitoneal injection to the rat. Age control rats received citrate buffer (0.3 mL) alone. Then DM was confirmed in 4 - 5 days after STZ injection and on the day of the experiment by using a glucose meter.

Maintenance of animals:

Control and diabetic rats were kept in separate cages and provided with feed and water for an average period of 4- 6 weeks until they were used in the study. Both groups were kept in a temperature-controlled room (22±2 °C) daily. The animals were fed with standard food and provided with water. After the initial weights of the rats were recorded, the weights were routinely checked and recorded once a week until the end of the study.

Preparation of rat urinary bladder strips:

Rats were humanely killed by a blow on their head followed by cervical dislocation. An incision made in the lower abdomen and urinary bladder was located and then urinary bladder was removed rapidly and placed in Krebs solution (composition in Millimolar (mM): NaCl - 119, KCl - 4.8, $MgCl_2$ - $6H_2O$ 1.2, KH_2PO_4 - 1.2, $CaCl_2$ - 2.5, $NaHCO_3$ - 25 and glucose - 10.0) whose pH was 7.4. The mucosa was removed by careful dissection and four longitudinal strips were isolated from the bladder body by making excisions on the body of the bladder on the mid-parts of the anterior and posterior surfaces.

Sample Preparation for the Measurement of Ions:

At the end of the experiment, the tissue were taken out of the organ bath, blotted, weighed and dissolved in 1 - 2 mL of concentrated nitric acid (HNO_3). Unstimulated tissues were also dissolved in concentrated HNO_3 for the measurement of ions experiment. Then a volume of 0.2 mL of the tissue sample solution was added to 9.8 mL of deionized water. This solution was vortex mixed and was used for the measurements of Na^+ and K^+ using flame photometry and for measurements of Ca^{2+}, Mg^{2+} using Flame Atomic Absorption Spectrophotometry (FAAS).

Measurement of Ions Using the Inductively Coupled Plasma- Mass Spectrometry:

Firstly, tissue sample, which were dissolved in 1mL concentrated nitric acid and diluted 50 times by the addition of 9.8 mL deionized water, were taken. Out of 10 mL the solution 1ml of sample was mixed with 4 ml of nitric acid (HNO_3) and 1 ppm of Cesium. Then this solution was vortex mixed and used for the measurements of Cu^{2+},

Zn^{2+}, Pb^{2+} and Fe^{2+}using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) (Fig. 2.6). The ICP-MS is a relatively young technique which allows the simultaneous detection of almost all elements of the periodic table. The main advantage of this method is the low detection limits. ICP-MS technique combines high temperature argon plasma (6000 to 8000°K) as a highly efficient ion source with a mass spectrometer.

The plasma is generated in a quartz torch. Solutions of samples are introduced into the plasma via a nebuliser as a fine aerosol. When the aerosol reaches the plasma the sample gets completely volatilised, atomised, and ionised under atmospheric pressure. This process produces a cloud of positively charged ions. The sample ions are transferred into a vacuum system containing a mass filter. In the analyser the ions are separated according to their mass-to-charge ratio (m/z). The ions are detected by a secondary electron multiplier. For quantitative analysis the count rate obtained for a certain ion is proportional to its concentration.

Statistical Analysis:

Results are presented as a Mean ± Standard Error of the Mean (SEM). SPSS software was used (SPSS for Windows, Version 15.0) to compare diabetic and age - matched control values using student independent sample t- test and student's paired t- test. A value less than 0.05 was considered significant ($P<0.05$) while a P value less than 0.01 ($P<0.01$) was considered highly significant in contrast, a P value greater than 0.05 was considered insignificant ($P>0.05$).

RESULTS AND DICUSSION:

I. General Characteristics

On arrival in the laboratory, the body weights of all rats were 164.109 ± 2.157 g (n=12) before induction of diabetes. When rats injected with Streptozotocin the weight of whole diabetic rat weighed significantly (*$P<0.05$) less than control rats. As was to be expected, the blood glucose levels in the STZ treated diabetic rats were significantly (*$P<0.05$) high when compared to the control rats (**Table 1**). Data is shown as S.E.M. n = 6 rats for each parameter and compared to age - matched control. Note the significant increase in (*$P<0.05$) in mean blood glucose, mean weight of bladder and the decrease (*$P<0.05$) in mean weight of diabetic rats. The mean blood glucose levels of the diabetic rats (28.68 ± 1.424 mM) (n=6) about four to five times higher than those of age-matched control (5.43 ± 0.258 mM) (n=6) rats. The mean weights of bladders of the streptozotocin-diabetic rats (0.369 ± 0.078 g) (n=6) were four time high than those of control rats (0.088 ± 0.033 g) (n=6). As well as the mean weight of bladder strips of the streptozotocin-diabetic rats (0.0855 ± 0.041 g) (n=6) were more than control rats (0.021 ± 0.024 g) (n=6).

The result of this study showed marked elevation in the sodium levels in both control unstimulated and stimulated strips of urinary bladder than both diabetic unstimulated and stimulated strips. These indicate that Na^+ pump activity is an important modulator of bladder smooth muscle tone, diabetes diminishes Na^+ pump activity and inhibits agonist-induced contractions in bladder and an increase inintracellular Na^+ concentration, secondary to inhibition of bladder smooth muscle Na^+ pump activity is associated with reduced responsiveness to contractile agonists. Diminished Na^+ pump activity in diabetes may be contributes to the development of bladder cystopathy and neuropathy [8].

TABLE 1: GENERAL CHARACTERISTICS AND EFFECTS OF CONTROL AND STZ-INDUCED DIABETES RATS.

Parameters	Units	Control	Diabetic
Mean Weight of Rat on arrival at the laboratory	G	164.109 (12) ±2.157	N/A
Mean Weight of Rat during experiment	G	375.383 (6) ± 9.353	*189.333 (6) ± 1.994
Mean Blood Glucose	mM	5.43 (6) ± 0.258	*28.68 (6) ± 1.424
Mean Weight of Bladder	G	0.088 (6) ± 0.033	*0.369 (6) ± 0.078
Bladder weight to body weight ratio	Ratio	0.023± 0.001	*0.190 ± 0.005
Mean Weight of Bladder Strip	G	0.021 (6) ± 0.024	*0.0855 (6) ± 0.041

II. Cation contents:

In order to measure the cation content in the control and diabetic urinary bladder without stimulations, the tissue were dissected from rats, weighted and then finally dissolve in concentrated nitric acid. **Fig. 1**and **2** shows the concentration of sodium and

magnesium respectively for stimulated and unstimulated control and diabetic urinary bladder. In **Fig. 1**, each bar is represented as the mean of 5 observations (n = 5) with the ± SEM shown as vertical bars. Note that control unstimulated has significantly (*P<0.05) more sodium than control stimulated, diabetic unstimulated and diabetic stimulated tissue strips while in **Fig. 2**, each bar is represented as the mean of 5 observations (n = 5) with the ± SEM shown as vertical bars. Note that control unstimulated has significantly (*P<0.05) more magnesium than control stimulated, diabetic unstimulated and diabetic stimulated tissue strips. **Fig.3, 4,5, 6,7** and **8** (**C, D, E, F, G** and **H**) shows the concentration of 3-potassium, 4-calcium, 5-zinc, 6-copper, 7-lead and 8- ferrous respectively for stimulated and unstimulated control and diabetic urinary bladder.

The results also show that the levels of cations for Na^+, K^+, Ca^{2+} and Mg^{2+} were significantly (*P<0.05) reduced in unstimulated diabetic urinary bladder than unstimulated control urinary bladder but for trace elements like Zn^{2+}, Cu^{2+} and Pb^{2+} the cations content increased in unstimulated diabetic urinary bladder than unstimulated control urinary bladder**.**

Magnesium is an important cation in all living cells being a cofactor of many enzymes, especially those utilizing high energy phosphate bounds. The relationship between insulin and magnesium has been recently studied. In control, it has been shown that magnesium plays the role of a second messenger for insulin action; on the other hand, insulin itself has been demonstrated to be an important regulatory factor of intracellular magnesium accumulation.

Conditions associated with insulin resistance, such as hypertension or aging are also associated with low intracellular magnesium contents. In diabetes mellitus, it is suggested that low intracellular magnesium levels result from both increased urinary losses and insulin resistance.

The extent to which such low intracellular magnesium content contributes to the development of macro- and microangiopathy remains to be established. Reduced intracellular magnesium content might contribute to the impaired insulin response and action which occurs in Type 2 diabetes mellitus.[9]

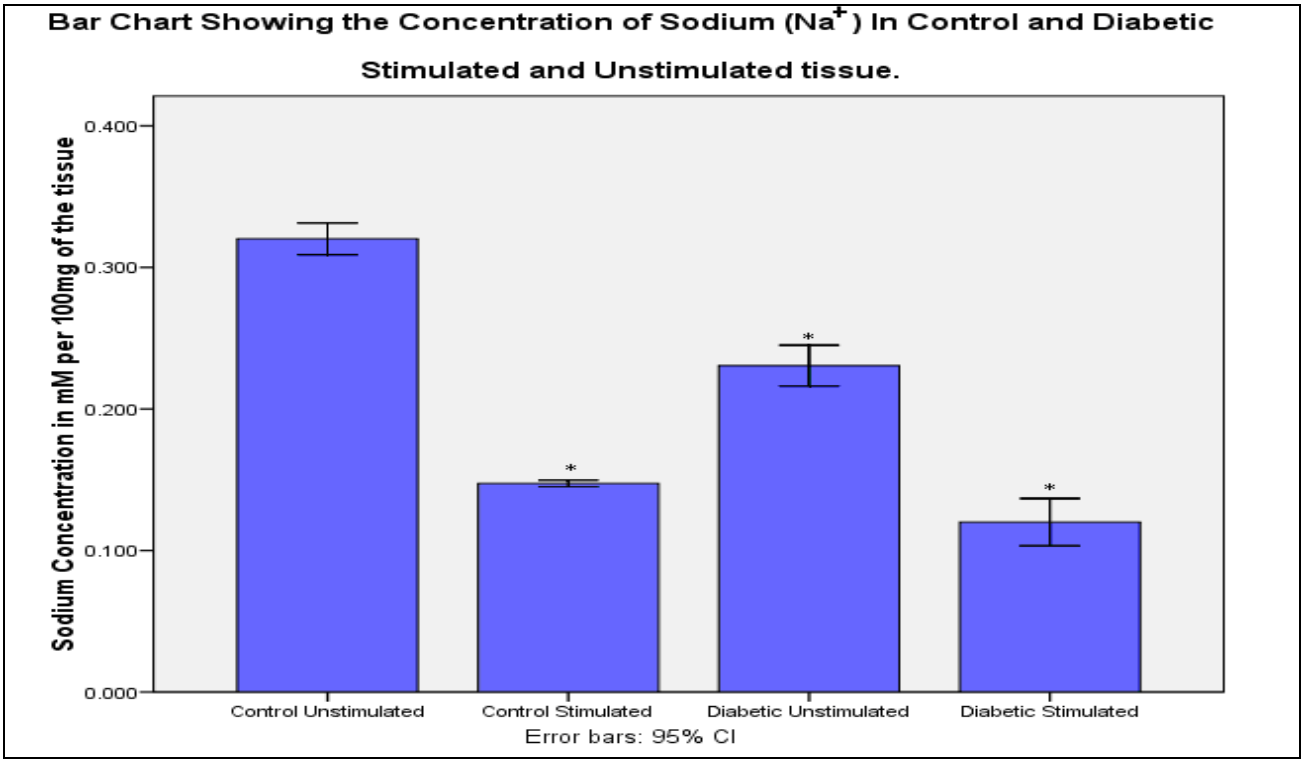

FIG.1: BAR CHARTS SHOWING THE CONCENTRATION OF SODIUM IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF FLAME PHOTOMETRY.

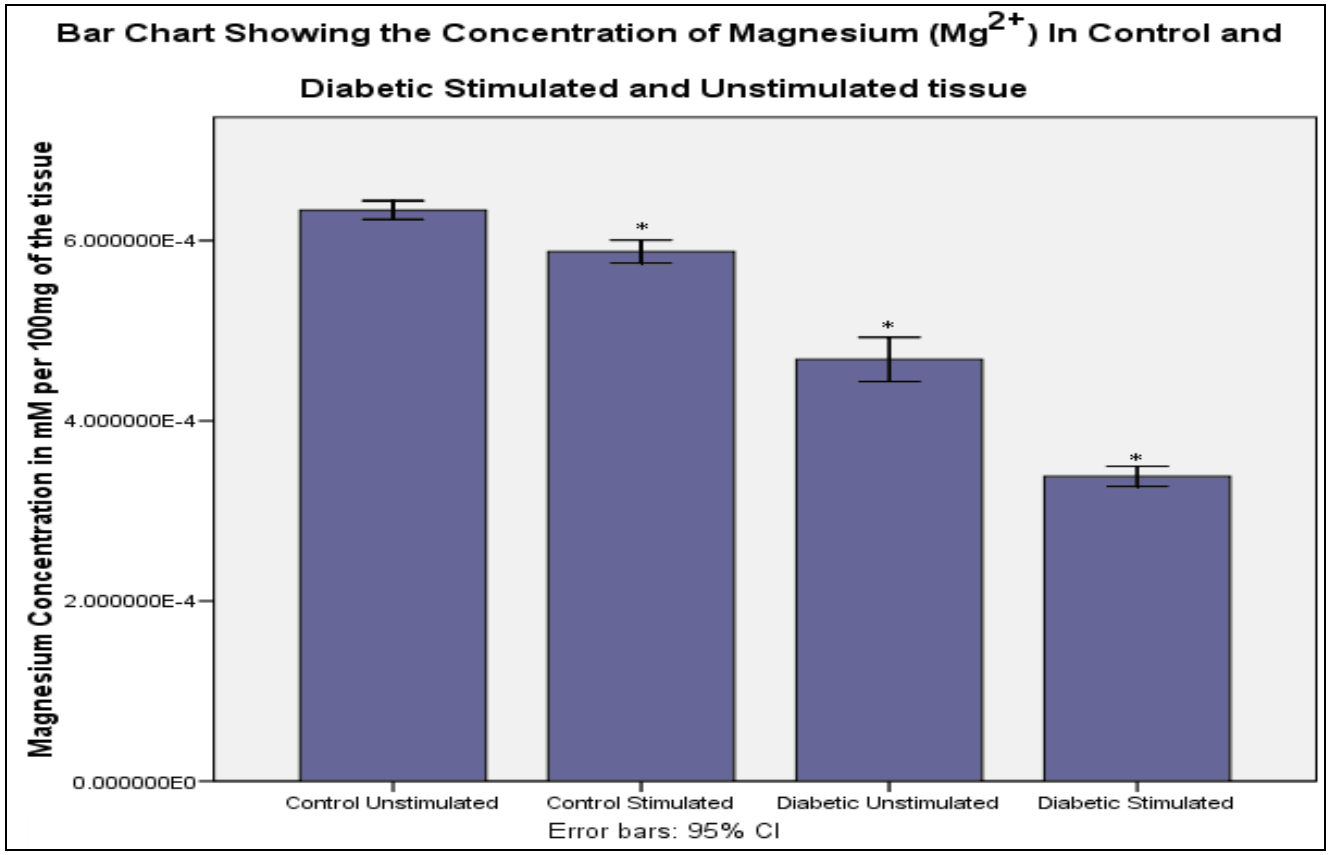

FIG.2: BAR CHARTS SHOWING THE CONCENTRATION OF MAGNESIUM IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF ATOMIC ABSORPTION SPECTROPHOTOMETRY.

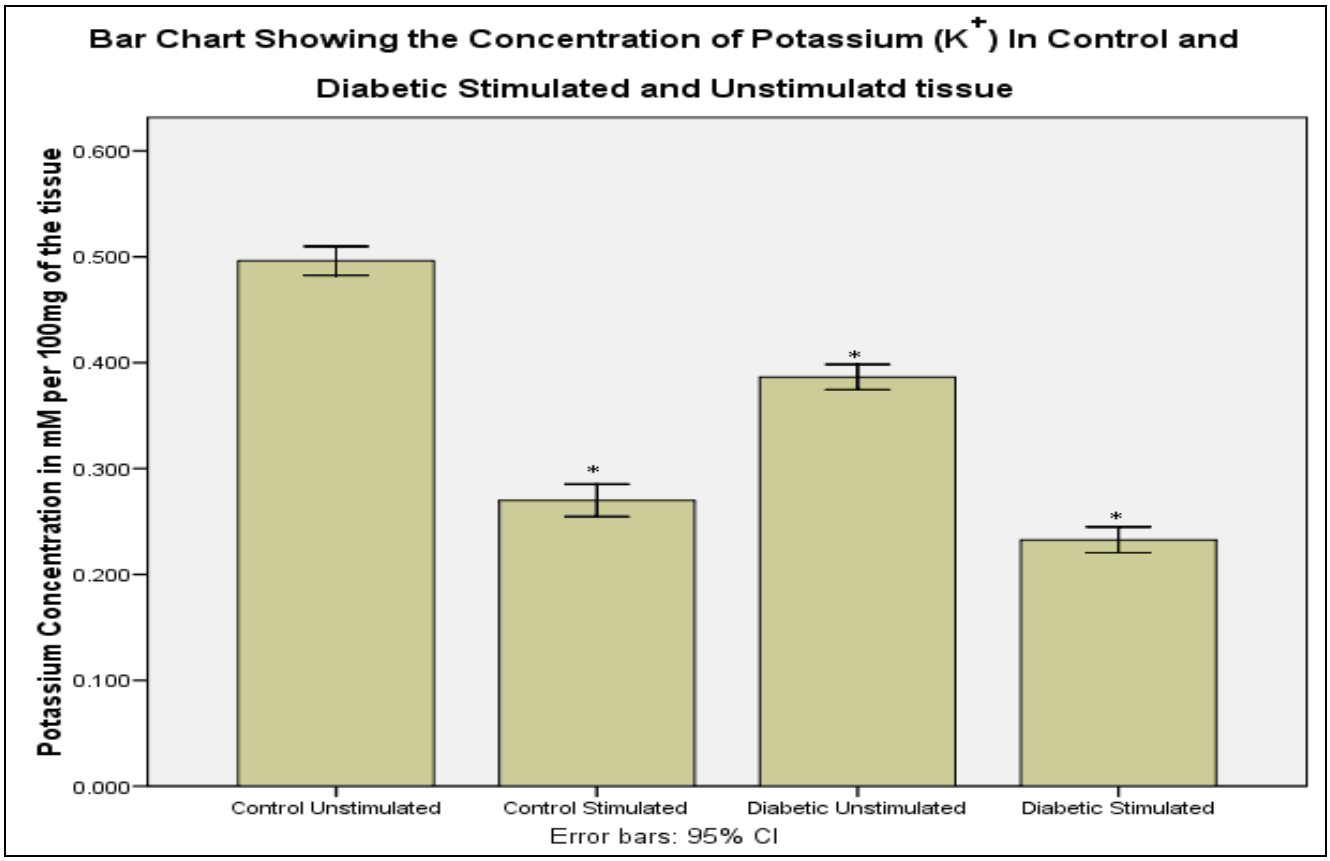

FIG.3: BAR CHARTS SHOWING THE CONCENTRATION OF POTASSIUM IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF FLAME PHOTOMETRY.

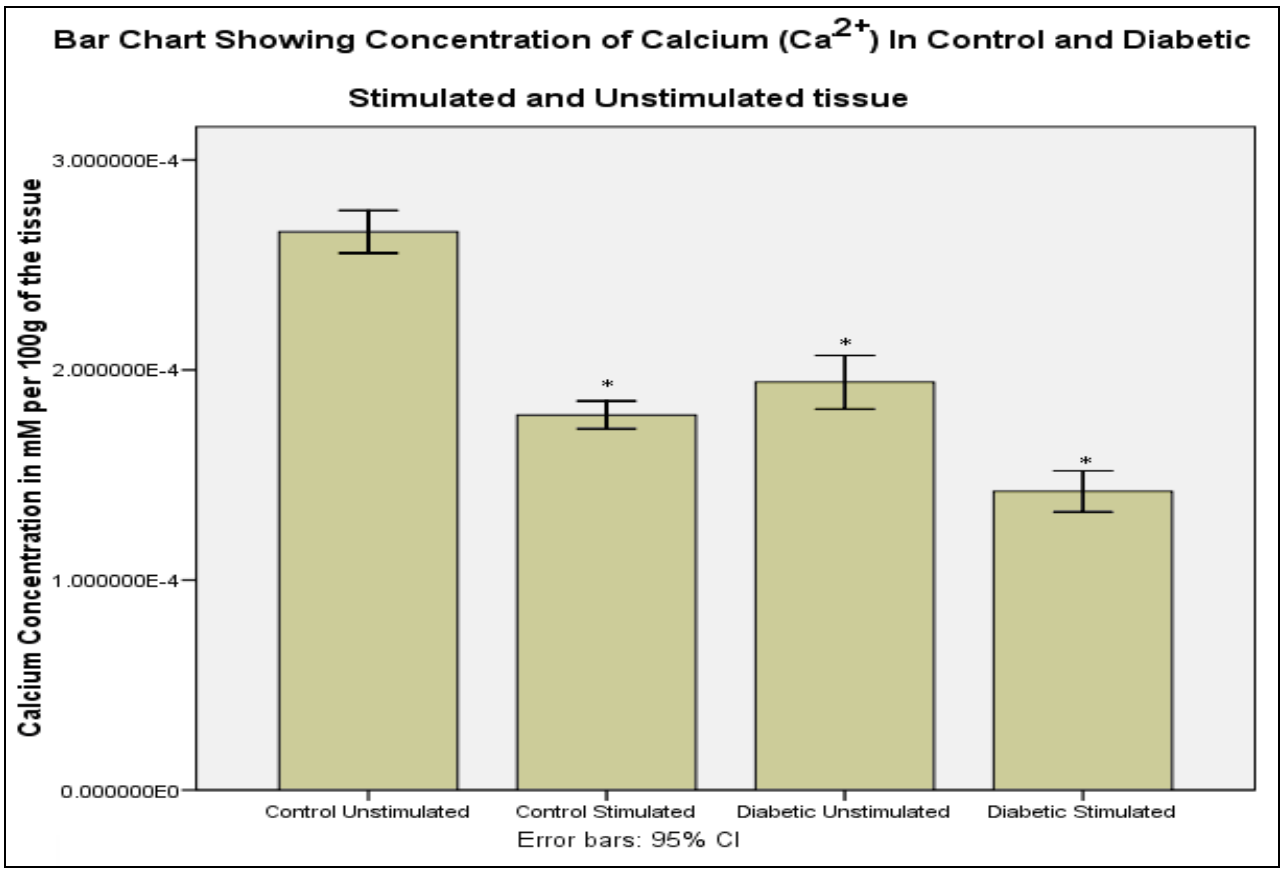

FIG.4: BAR CHARTS SHOWING THE CONCENTRATION OF CALCIUM IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF ATOMIC ABSORPTION SPECTROPHOTOMETRY.

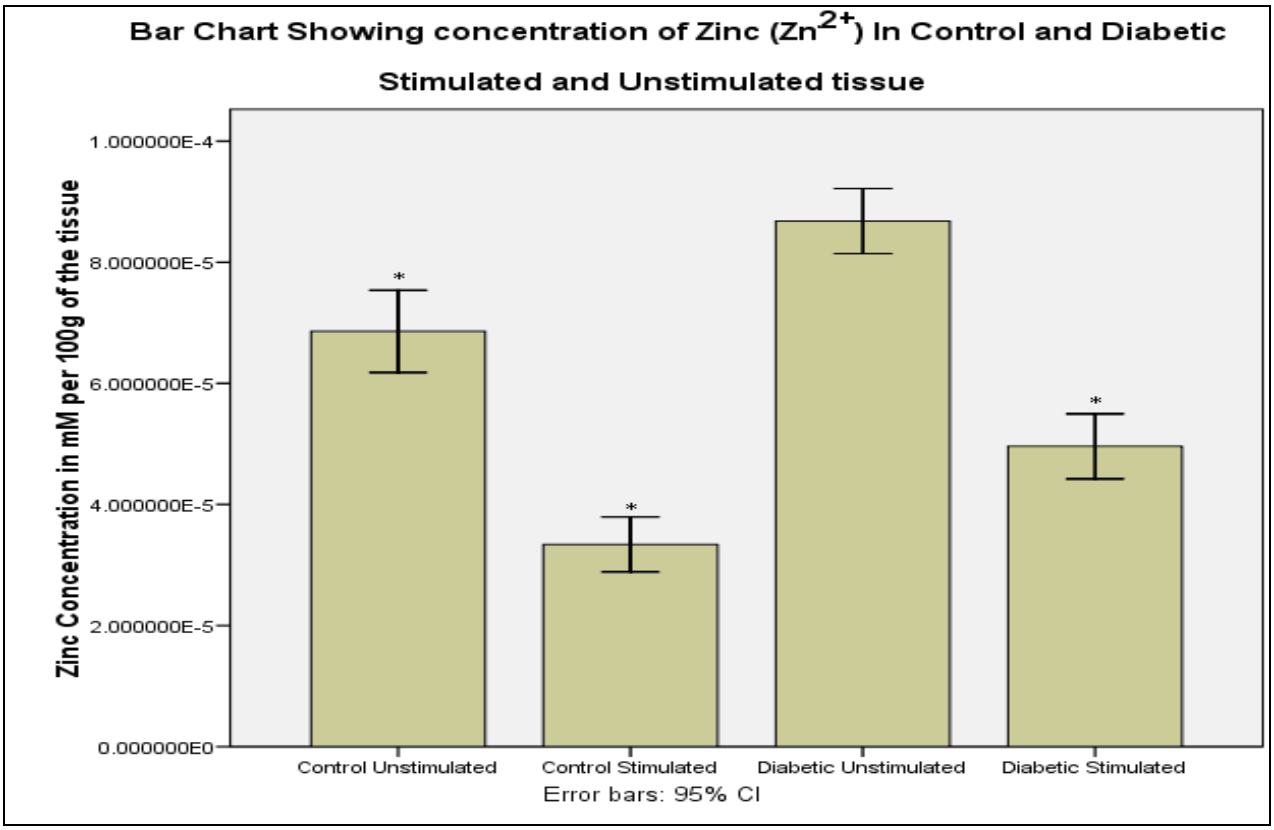

FIG.5: BAR CHARTS SHOWING THE CONCENTRATION OF ZINC IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY.

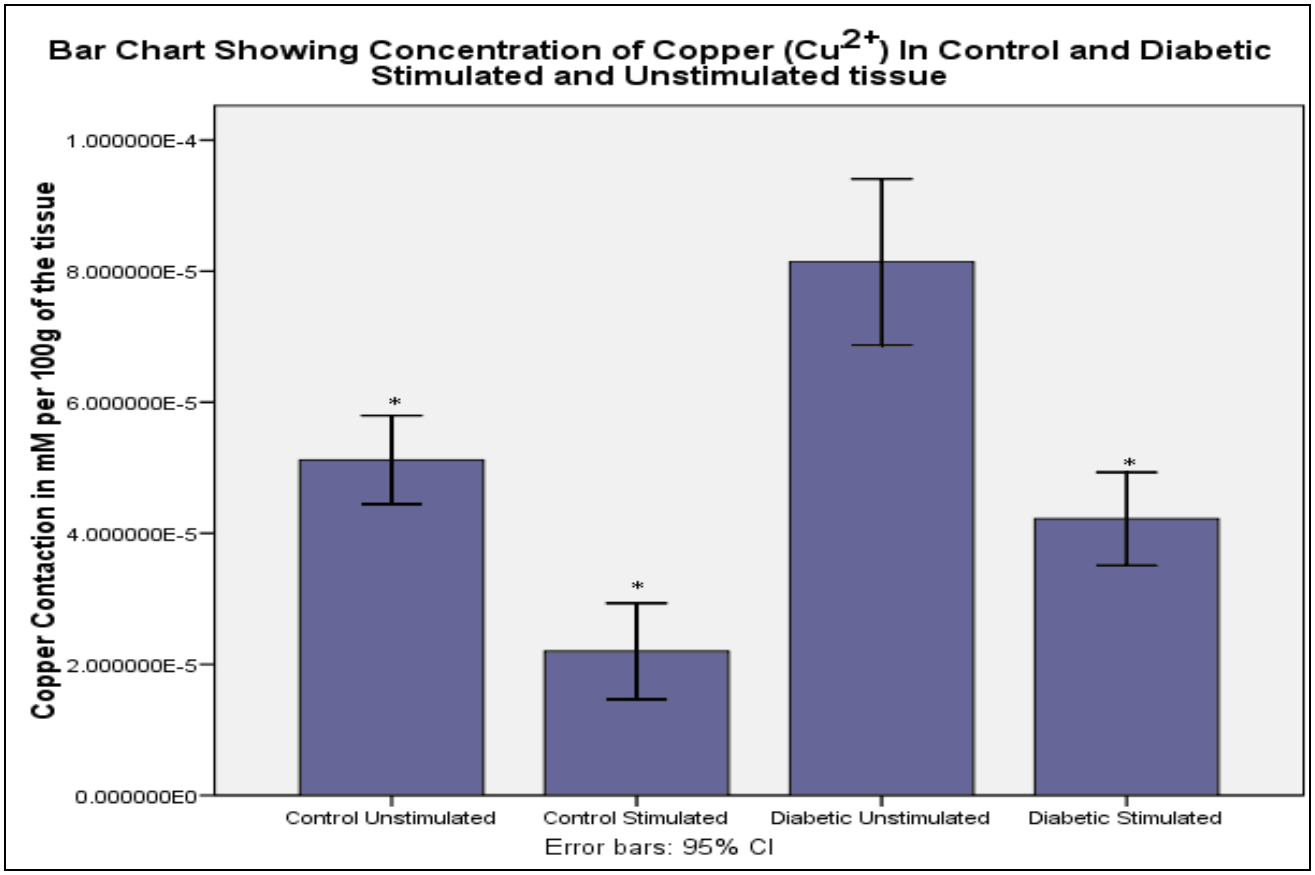

FIG. 6: BAR CHARTS SHOWING THE CONCENTRATION OF COPPER IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY.

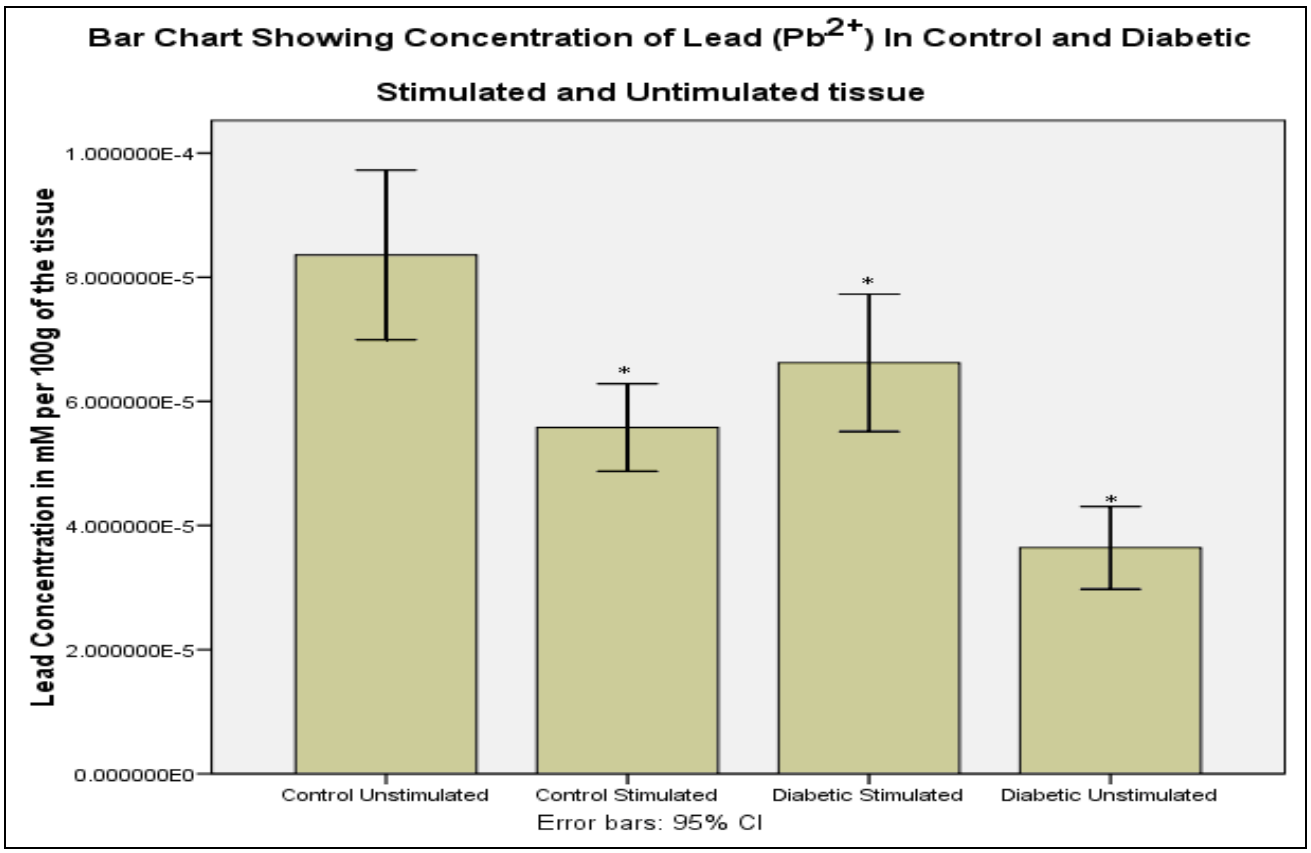

FIG.7: BAR CHARTS SHOWING THE CONCENTRATION OF LEAD IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY.

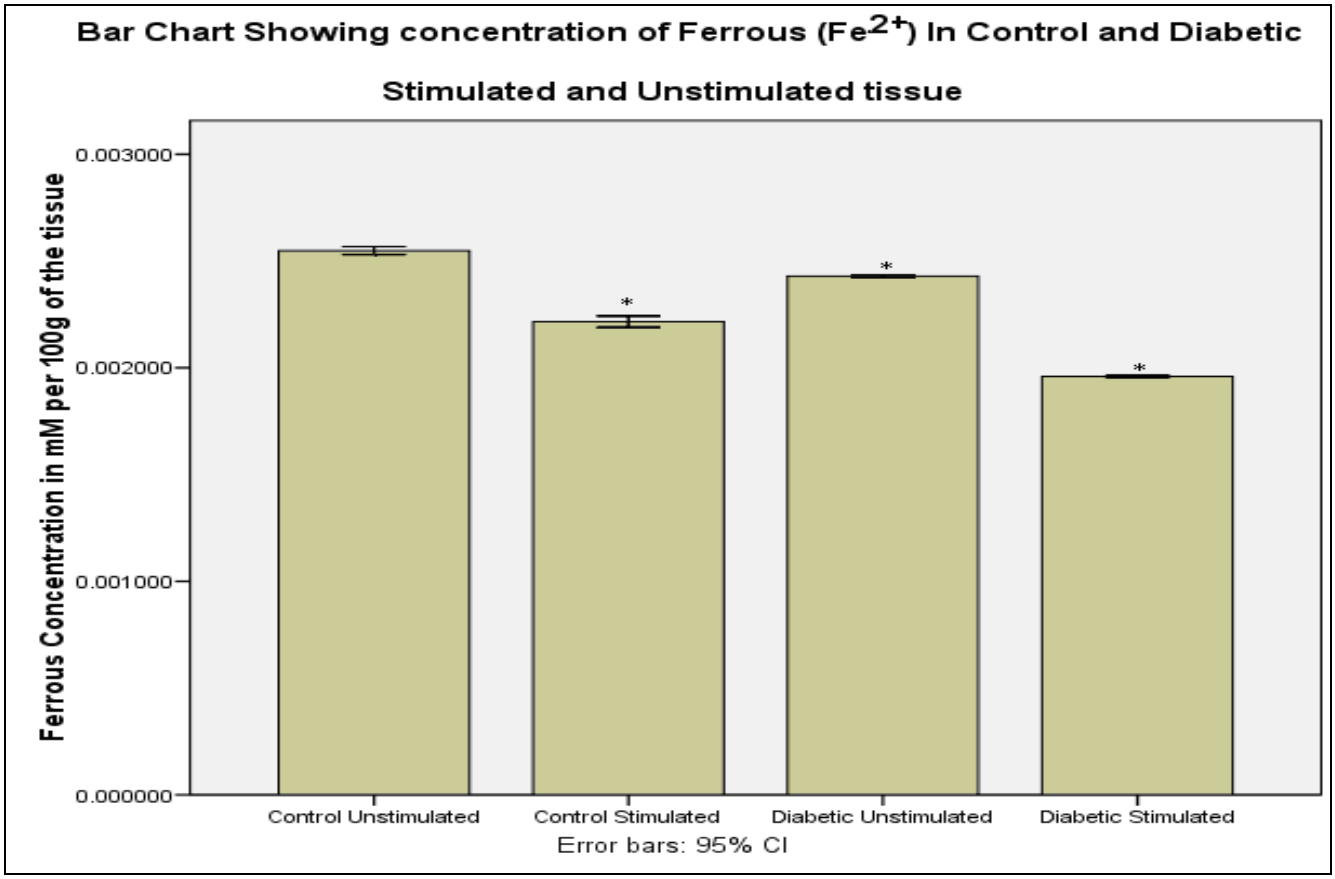

FIG.8: BAR CHARTS SHOWING THE CONCENTRATION OF FERROUS IN CONTROL AND DIABETIC STIMULATED AND UNSTIMULATED TISSUE DETERMINED BY USE OF INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY.

The result of this study showed marked elevation in the potassium levels in both control unstimulated and stimulated strips of urinary bladder than both diabetic unstimulated and stimulated strips. These results suggest that there is normal permeability or the function of Na^+-K^+ pump in control strips while in diabetes strips there is small changes in membrane permeability or in the function of Na^+-K^+ pump and due to this bladder obstruction produced in diabetes. [10] The total Ca^{2+} concentrations in STZ induced rats decreased as compared to the age- matched control animals. Previous reports suggest that there is a decrease in Ca^{2+} levels in the tissue due to decreased in the Ca^{2+} influx into detrusor muscle in DM as well the L-type Ca^{2+} channels are impaired during DM which cause reduction in Ca^{2+} influx. As also the Na^+ /Ca^{2+} exchanger pump working to shunt Ca^{2+} out of the cell is decreased in DM urinary bladder as compared to control. [11]

The result of this study showed marked elevation in the zinc levels in both diabetic unstimulated and stimulated strips of urinary bladder than both control unstimulated and stimulated strips. These results suggest that Zn^{2+}plays a clear role in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin in the hexameric form. But in diabetes the increased Zn^{2+}affects the ability of the islet cell to produce and secrete insulin. As the disease progresses, there may be an exhaustion of the beta cells with a relative inability to keep up with the needs for insulin production.

The resultant decrease in insulin produced results in even greater hyperglycemia. When the rats were exercised, which allows insulin independent glucose transport in muscle, tissue Zn^{2+}decreased suggesting zinc may be lost from cells as glucose is translocated into muscle. Other studies also suggest that these same tissues increase zinc concentrations in the diabetic state.[12, 13] The result of this study showed marked elevation in the zinc levels in both diabetic unstimulated and stimulated strips of urinary bladder than both control unstimulated and stimulated strips. STZ administration decreases the ability of rat to synthesize and secrete insulin by

destroying β-pancreatic cells in a dose-dependent manner. The resultant decrease in insulin produced results in even greater hyperglycemia. [14] The total lead levels in STZ-induced urinary bladder are reduced as compared to control. Low levels of Pb^{2+}decrease the capacity of urinary bladder mitochondrion to produce ATP and thus decrease Ca^{2+}out flux leading to malfunctioning of bladder.[15]

CONCLUSION: The present results show that DM is generally associated with significant physiochemical changes in urinary bladder tissues with regards to body weight, blood glucose and cation levels when compared to controls. Based on the data, it has been speculated that diabetes may induce changes in urinary bladder tissues and changes in Na^{+}/K^{+} channel function. Hence we conclude that these differences in the cation contents in STZ-induced DM results in malfunctioning of bladder and the development of long term complications of DM.

ACKNOWLEDGEMENT: The authors want to acknowledge University of Central Lancashire, UK staff for research support.

CONFLICT OF INTEREST: There is no conflict of interest in present work.

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How to cite this article:
Sangar VC and Singh J: Effects of Streptozotocin- Induced Type I Diabetes Mellitus on Cation Contents in Urinary Bladder Tissues of the Rat. Int J Pharm Sci Res 2016; 7(2): 789-97.doi: 10.13040/IJPSR.0975-8232.7(2).789-97.